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FORM PTO-1390 (Modified) (REV 5-93) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY'S DOCKET NUMBER <u>065691/0179</u>
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371		U S APPLICATION NO. (If known, see 37 CFR 1.57) <u>09/462909</u> Unassigned
INTERNATIONAL APPLICATION NO. PCT/FR98/01556	INTERNATIONAL FILING DATE July 16, 1998	PRIORITY DATE CLAIMED July 16, 1997
TITLE OF INVENTION NOVEL PEPTIDES AND POLYPEPTIDES USEFUL FOR REGENERATING THE NERVOUS SYSTEM		
APPLICANT(S) FOR DO/EO/US Annie MEINIEL, Hubert MONNERIE and Stephane GOBRON		
Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:		
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371.</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT Articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2)) <input type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau). <input checked="" type="checkbox"/> has been transmitted by the International Bureau. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)) <input type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau). <input type="checkbox"/> have been transmitted by the International Bureau. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired. <input checked="" type="checkbox"/> have not been made and will not be made.</p> <p>8. <input type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input checked="" type="checkbox"/> A copy of the translation of annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p>		
Items 11. to 16. below concern other document(s) or information included:		
<p>11. <input type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: Paper copy of Sequence Listing</p>		

U.S. APPLICATION NO. known, see 37 CFR 1.30 Unassigned	INTERNATIONAL APPLICATION NO PCT/FR98/01556	ATTORNEY'S DOCKET NUMBER 065691/0179				
17. <input checked="" type="checkbox"/> The following fees are submitted:		CALCULATIONS PTO USE ONLY				
Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO \$840.00						
International preliminary examination fee paid to USPTO (37 CFR 1.482) \$670.00						
No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) \$690.00						
Neither international preliminary examination fee (37 CFR 1.482) nor International search fee (37 CFR 1.445(a)(2)) paid to USPTO \$970.00						
International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) \$96.00						
ENTER APPROPRIATE BASIC FEE AMOUNT =		\$840.00				
Surcharge of \$130.00 for furnishing the oath or declaration later than 20 Months from the earliest claimed priority date (37 CFR 1.492(e))						
Claims	Number Filed	Included in Basic Fee	Extra Claims	Rate		
Total Claims	17	-	20	= 0	x \$18.0	\$0.00
Independent Claims	1	-	3	= 0	x \$78.0	\$0.00
Multiple dependent claim(s) (if applicable)					\$260.0	
TOTAL OF ABOVE CALCULATIONS =					\$840.00	
Reduction by ½ for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).					\$0.00	
SUBTOTAL =					\$840.00	
Processing fee of \$130.00 for furnishing English translation later the 20 months from the earliest claimed priority date (37 CFR 1.492(f)).				+		
TOTAL NATIONAL FEE =					\$840.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property +						
TOTAL FEES ENCLOSED =					\$840.00	
				Amount to be: refunded	\$	
				charged	\$	
a. <input checked="" type="checkbox"/>	A check in the amount of \$840.00 to cover the above fees is enclosed.					
b. <input type="checkbox"/>	Please charge my Deposit Account No. <u>19-0741</u> in the amount of \$840.00 to the above fees. A duplicate copy of this sheet is enclosed.					
c. <input checked="" type="checkbox"/>	The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>19-0741</u> . A duplicate copy of this sheet is enclosed.					
NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.						
SEND ALL CORRESPONDENCE TO:						
Foley & Lardner Washington Harbour 3000 K Street, N.W., Suite 500 Washington, D.C. 20007-5109			SIGNATURE			
			NAME	PATRICIA D. GRANADOS		
REGISTRATION NUMBER 33,683						

NOVEL PEPTIDES AND POLYPEPTIDES USEFUL FOR
REGENERATING THE NERVOUS SYSTEM

The present invention relates in particular to novel peptides and polypeptides useful in particular as medicines in therapeutic treatments involving the regeneration of the nervous system cells, for treating neuroblastomas, and also useful as additives in the cultures of nerve cells.

Many proteins comprising repeating units which are called thrombospondin type I units (TSRs) have been identified during the past few years. It can be said that these proteins have highly varied activities depending on the biological system in which they are involved. There may be mentioned, as the best studied and therefore the best known examples, the CS proteins (of circumsporozoite) which allow binding to the hepatic cells of the agent for the propagation of malaria, the plasmodium falciparum sporozoite (WO 94/06646) and the thrombospondin secreted by the blood platelets which are involved in the phenomena of thrombosis and angiogenesis (EP 443 404).

In fact, this thrombospondin type 1 unit (TSR) comprises, in all the proteins studied so far and previously mentioned, about 60 amino acids (AA) some of which, like cysteines (C), tryptophans (W), serines (S), glycines (G), arginines (R) and prolines (P) are highly conserved (see below the alignment of these conserved AAs in a few proteins).

Some synthetic peptides, deduced from these TSR units, have valuable biological properties. Thus, the CSVTCG units allow the adhesion of the plasmodium sporozoites to the hepatic cells, the CSVTCG and WXXW units allow cellular attachment in other biological models, BBXB (B being a basic amino acid) binds heparin and finally WSXWS binds certain growth factors.

F-spondin has been described and its sequence has been aligned with that of thrombospondin in Klar et al., (1992), Cell, 69, 95-110.

The general characteristics of SCO-spondin are described in particular in the article by Monnerie et al. (submitted) and the article by Gobron et al., (1996), Journal of Cell Science, 109, 1053-1061, 1996.

5 In particular, the alignment of the sequence of SCO-spondin has revealed homologies with proteins such as thrombospondin 1 and 2 (see sequence, alignment page 1057 of Gobron et al., (1996), J. of Cell Science 109, 1053-1061, incorporated into the description by reference).

10

The novelty of the present invention consists in the identification and the selection of a novel peptide which is active in the regeneration of the nervous system, whose sequence is derived from one of 15 the TSRs of SCO-spondin.

More particularly, the present invention relates to a peptide or polypeptide having the formula:

-W-S-A₁-C-S-A₂-C-G- (SEQ ID No. 1)

20

in which A₁ and A₂ are amino acid sequences comprising 1 to 5 amino acids, with the exception of the peptides or polypeptides having one of the following sequences

25

-W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

30

It should be recalled that in the description as a whole, "amino acid" is understood to mean both the natural amino acids and the non-natural amino acids. "Natural amino acid" is understood to mean the amino acids in the L form which can be found in natural proteins, that is to say alanine, arginine, asparagine, 35 aspartic acid, cysteine, glutamine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine and valine. However, the present invention also relates to the non-natural amino acids,

that is to say the preceding amino acids in their D form, as well as the homo forms of some amino acids such as arginine, lysine, phenylalanine and serine or the nor forms of leucine or valine.

5 It is also possible to envisage using other amino acids such as, for example:

Abu : alpha-aminobutyric acid

Agm : agmatine

Aib : alpha-aminoisobutyric acid

10 F-trp : N-formyl-trp

sarcosine

statine

ornithine

desaminotyrosine.

15 Desaminotyrosine is incorporated at the N-terminal end whereas agmatine and statine are incorporated at the C-terminal end of these peptides.

20 Preferably, the peptides according to the present invention A₁ is proline or X₁-W-X₂-X₃ (SEQ ID No. 5) where X₁, X₂, X₃ are chosen, independently of each other, from G, S and C, that is to say small amino acids.

Still preferably, A₁ is X₁-W-S-X₃ (SEQ ID No. 6) and A₂ is chosen from RS, VS and VT.

25 The reasons for these choices will emerge on reading some examples.

Preferably, the polypeptide according to the present invention has the following structure:

-W-S-X₁-W-S-X₂-C-S-A₂-C-G- (SEQ ID No. 7)

30 The preferred peptide has the following structure:

-W-S-G-W-S-S-C-S-R-S-C-G- (SEQ ID No. 8)

35 Preferably, the peptides and polypeptides according to the present invention will have the following structure:

Y-W-S-A₁-C-S-A₂-C-G-Z (SEQ ID No. 9)

in which Y and Z constitute the N- and C-terminal ends of the peptide, or comprise amino acid chains having

less than 6 amino acids, or comprise chains of compounds which are not amino acids.

This corresponds to the peptide per se or to a peptide in which the Z and Y ends enhance the pharmacological activity or ensure a better penetration or bioavailability of the active ingredient; thus, it is possible to envisage in the Y and Z ends the use of hydrophilic components which make it possible, where appropriate, to cross certain biological barriers, or alternatively, on the contrary, to envisage more hydrophilic sequences which will allow a better solubilization of the products involved.

Finally, the modification of the ends can facilitate the incorporation of these products into particular galenic forms such as, for example, liposomes or microparticles.

The present invention also relates to DNA expression vectors characterized in that they are capable of expressing the preceding peptides or polypeptides.

The DNA sequences encoding the preceding peptides or polypeptides can be easily determined from amino acid sequences or based, for example, on the natural sequences as will be described in the present application.

The vectors for administration may consist of naked DNA vectors, plasmid vectors, viral vectors or alternatively synthetic vectors.

These are known technologies which will not be described in detail.

The use of these expression vectors makes it possible to express *in situ* the peptides or polypeptides involved and, in some cases, is likely to enhance their activity.

Constructs will of course be chosen which exhibit, if possible, specificity for the nerve cells, since they are the preferred targets for the polypeptides according to the present invention.

The peptides and polypeptides according to the present invention may be prepared by any appropriate method, in particular they may be obtained by chemical synthesis, but it is also possible to obtain them by 5 the biological route using in particular the vectors mentioned above in appropriate cell cultures.

It should in fact be noted, in this regard, that the polypeptides and peptides according to the present invention may be provided in deglycosylated or 10 glycosylated form if necessary. It should also be noted that in some cases and depending on the method of preparation, it may be necessary to renature some tertiary structures of the peptide.

Finally, the polypeptides according to the 15 present invention can be more particularly used for the manufacture of a medicine with the aim of being administered *in vivo*, in particular in all pathological conditions and traumas requiring regeneration of the nervous system cells, and more particularly of their 20 outgrowths and synapses.

These may be pathological conditions or traumas in which neurodegeneration is observed, but they may also be pathological conditions or traumas in which the 25 regeneration of the central nervous system, in particular of the axons, or of the peripheral nerves is necessary.

Among the neurodegenerative pathological conditions in which the compounds according to the present invention may provide a support, there may be 30 mentioned in particular Alzheimer's disease, multiple sclerosis, Parkinson's disease and the different types of myopathies.

As regards the regeneration of the neuronal outgrowths, in particular of the axons, this may 35 involve in particular accident- or trauma-type problems (section of the spinal cord or of the peripheral nerves).

Likewise, the compounds according to the present invention may be used as additives in certain

cell cultures with the same effects as those mentioned above on the growth of cells.

More particularly, the compounds according to the present invention increase neuritic growth (including the axons) in the cerebral cortex neurons. Inhibition of aggregation and defasciculation of the neurites are noted on the spinal cord neurons and an increase in synaptic contacts is also noted.

"Neuritic growth" is defined as an extension, that is to say growth of the neuron outgrowths, whether the dendritic or axonal outgrowth.

"Aggregation" is defined as a grouping together of the cells forming a cluster.

"Defasciculation" is defined as the result of a decrease in adhesivity between neurites, leading to a loose network of neuronal outgrowths.

"Synaptic contact" is defined as the capacity for a neuronal cell to communicate with another cell, it being possible for the latter to also be neuronal.

In another aspect of the present invention, said peptides or polypeptides may be useful for inducing regression of tumorigenicity during a neuroblastoma.

The nomenclature used to describe the sequence of the present peptide is the international nomenclature using the three-letter code or the one-letter code and where the amino-terminal end is presented on the left and the carboxy-terminal end is presented on the right.

The compositions according to the present invention may be provided in any customary form for pharmaceutical administration, that is to say for example forms for liquid administration in a gel or any other support allowing, for example, controlled release.

Among the compositions which may be used, there should be mentioned in particular the injectable compositions more particularly intended for injections into the meningeal and subarachnoidal spaces.

The most active peptide according to the present invention has the following formula:

5 Trp-Ser-Gly-Trp-Ser-Ser-Cys-Ser-Arg-Ser-Cys-Gly
(SEQ ID No. 8)

It is soluble in basic aqueous medium, has a molecular weight of 1301 Da and has an amino acid composition of:

10

		N	N (%)	MW	MW (%)
C	Cys Cysteine	2	16.7	206	15.8
G	Gly Glycine	2	16.7	114	8.8
R	Arg Arginine	1	8.3	156	12.0
S	Ser Serine	5	41.7	435	33.4
W	Trp Tryptophan	2	16.7	372	28.6

It was obtained by solid phase chemical synthesis.

However, as was indicated above, it can be 15 obtained by genetic engineering using a host-vector system comprising DNA encoding the peptide taking into account, for example, the degeneracy so as to produce it in a large quantity.

The cDNA sequence encoding the peptide may be 20 presented in the following manner (SEQ ID No. 10):

5' TGG WSN GGN TGG WSN WSN TGY WSN MGN WSN TGY GGN 3'

A = Adenosine W = A or T
25 C = Cytosine S = G or C
G = Guanosine Y = C or T
T = Thymidine M = A or C
N = A, C, G or T

30 The peptide thus obtained was identified by microsequencing, HPLC analysis, mass spectrometry and sequencing of the complementary DNA.

It is on this peptide (SEQ ID No. 8) that the experiments described below were carried out.

5 Example 1 : effect of the peptide SEQ ID No. 8 on the growth of the neurons

Materials and method

10 Dissociated cell cultures of cerebral hemispheres of 8-day old chicken embryos

The neuronal cultures are obtained from 8-day old chicken embryos. The cerebral hemispheres, after removing the meninges, are cut into small pieces and enzymatically dissociated with 0.25% of trypsin in a 15 PBS saline buffer free of calcium and of magnesium for 15 minutes at 37°C.

20 The cells are centrifuged at 200 g for 5 minutes in DMEM medium with 20% FCS for the trypsin inactivation. The cells are then filtered on nylon membrane (pore size: 48 microns) and collected in a chemically defined medium free of serum containing a 1/1 mixture of DMEM and Ham's F12 medium supplemented with glutamine (4 mM), glucose (33 mM), penicillin G (50 U/ml), streptomycin sulfate (50 µg/ml) and an N2 supplement of Bottenstein and Sato (1979): putrescine (100 µM), sodium selenite (30 nM), human transferrin (50 µg/ml), progesterone (20 nM), insulin (5 µg/ml) and β-estradiol (1 pM). All the N2 supplements were bought from Sigma.

30 The cells are plated at a density of 7.5×10^4 cells/cm² on 24-well plastic plates. For some experiments, the plastic plates are coated either with fibronectin (24 µg/ml) or with thrombospondin (20 µg/ml). The cultures are incubated at 37°C and under air containing 10% CO₂. The medium is not changed during the experiment. These cultures consist of nearly 95% of neurons.

Cell cultures of spinal neurons

The spinal cords of 6-day old chicken embryos are dissected, freed of their meningeal membrane and cut into small pieces in a phosphate buffer (PBS) free of calcium and of magnesium. After incubation with 0.25% trypsin for 10 minutes at 37°C, the tissue is centrifuged at 200 g for 4 minutes in a growth medium containing 20% fetal calf serum in order to stop the trypsinization. The cells are then dissociated by repeated trituration using a Pasteur pipette and resuspended in a chemically defined medium free of serum as above.

The cells are plated at a density of 7.5×10^4 cells/cm² on 24-well plastic culture plates. The cultures are incubated at 37°C and under air containing 10% CO₂. The medium is not changed during the experiments and it has already been shown that this type of cell population contained more than 93% of neurons.

The peptides tested are, in addition to the peptide according to the present invention mentioned above (peptide SEQ ID No. 8), a second peptide according to the invention having the structure:

W-G-P-C-S-V-S-C-G- (SEQ ID No. 11)

then 3 peptides for comparison:

D-C-K-D-G-S-D-E- (SEQ ID No. 12)

R-K-A-R- (SEQ ID No. 13)

and a mixed sequence of the peptide SEQ ID No. 8:

S-S-C-R-S-G-C-W-G-S-S-W- (SEQ ID No. 14).

All these peptides were obtained by synthesis.

Results

In the presence of the peptide SEQ ID No. 8, the neurons aggregate and are essentially connected by bundles of long and thick neurites after 5 days of culture. Furthermore, these cells adhere well to the substrate coated with the peptide with no detachment of the aggregates. By contrast, the control cell cultures, in the absence of the peptide, rapidly detach from the plastic substrate at 5 days of culture. However, on

plastic, only the cortical neurons form aggregates from which very few neurites can be observed, which indicates that the substrate is insufficiently adhesive. The number of neuronal aggregates increases 5 by 9.3% between the control culture and the culture treated with the peptide according to the invention.

Morphometric analysis reveals a significant increase both in the number of neurites per aggregate and in the length of the neurites per aggregate. 10 Moreover, wells of plastic coated with BSA are only very slightly adhesive for the neuronal cells.

The tests carried out with other peptides in comparison with the peptide SEQ ID No. 8 at random give no significant result.

15 The peptide SEQ ID No. 11 gives lower but, nevertheless, significant results.

Likewise, the tests carried out with the peptide SEQ ID No. 13, which is a consensus sequence for attachment of glycosaminoglycans which is present 20 in a large number of proteins which bind to heparin, as well as the peptides corresponding to type A LDL receptors, gave no representative result.

Moreover, the effect of the peptides according 25 to the present invention SEQ ID No. 8 and No. 11 on cultures at low density was studied. Indeed, it has already been demonstrated that high aggregation could influence neuritic growth in the same manner as the strength of adhesion of the cells to the substrate.

The tests carried out at low density showed 30 that in the absence of aggregation, the two peptides significantly increased the percentage of neuronal cells carrying neurites. In the controls, only 24.4% of the adherent cells had neurites at 4 days of culture whereas 2 and 2.5 times as many appeared in the 35 presence of the peptides SEQ ID No. 8 and No. 11, respectively.

The morphometric analyses revealed a significant increase in each of them both in the number of neurites per cell and the length of the neurites in

the presence of the peptide SEQ ID No. 8 and not the peptide SEQ ID No. 11. Under these conditions, this demonstrates that, even in the absence of neuronal aggregation, the peptide SEQ ID No. 8 and to a lesser degree the peptide SEQ ID No. 11 are capable of promoting the adhesion and the neuritic growth of the cortical neuronal cells.

The effect of the peptide SEQ ID No. 8 of the invention was also studied under various experimental conditions:

In the presence of various substrates, it was possible to demonstrate, for example, that the peptide according to the invention significantly increased the number of neurites per aggregate in well-containing plates coated with thrombospondin and fibronectin, compared with the controls, as well as the length of the neurites per aggregate.

The activity of the peptide SEQ ID No. 8 on the spinal cord cell cultures compared with controls shows that the neurons remain distributed for at least one week *in vitro*. The neurons show prominent neuritic growths forming a network without fasciculation of the neurites. An increase in the number of synaptic contacts between the neurites is observed. By contrast, the neuronal cells of the controls form, in general, small aggregates interconnected by long filaments. The neurites growing from the aggregates form relatively rigid bundles along which essentially simple, bi- or tripolar neurons can be seen.

The other peptides tested under the same conditions show no notable difference compared with the controls.

Example 2 : Effect of the peptide SEQ ID No. 8 on the neuroblastoma derived from NIB104

Materials and method

The cells derived from the NIB104 neuroblastoma were cultured in 24-well plastic plates previously

coated with a film of poly-L-lysine, under conditions similar to those for the primary cultures.

Results

5 In the presence of the peptide SEQ ID No. 8 according to the present invention, the NIB104 neuroblastoma cells are considerably less numerous than in the control cultures. The appearance of the cells is considerably modified because they acquire a
10 characteristic neuronal phenotype. Morphometric analysis reveals that in the presence of increasing concentrations of peptide in the culture medium, the neuritic growth gradually increases. This response is therefore dose-dependant and indicative of a specific
15 physiological effect.

- 13 -

CLAIMS

1. Peptide having at least the following amino acid sequence:

5 -W-S-A₁-C-S-A₂-C-G- (SEQ ID No. 1)

in which A₁ and A₂ are amino acid sequences comprising 1 to 5 amino acids with the exception of the peptides or polypeptides having one of the sequences:

-W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

10 -W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

-W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

15 -W-S-Q-C-S-V-Y-C-G

-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- and T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N.

2. Peptide according to Claim 1, characterized in that A₁ is Pro or -X₁-W-X₂-X₃- (SEQ ID No. 5), X₁, X₂ and X₃ being chosen, independently of each other, from G, S and C.

3. Peptide according to Claim 2, characterized in that A₁ is X₁-W-S-X₃ (SEQ ID No. 6).

4. Peptide according to one of Claims 1 to 3, characterized in that A₂ is chosen from: R-S, V-S and V-T.

5. Peptide according to one of Claims 1 to 4, characterized in that it comprises at least the sequence -W-S-X₁-W-S-X₂-C-S-A₂-C-G- (SEQ ID No. 7).

30 6. Peptide according to one of Claims 1 to 5, characterized in that it is:

-W-S-G-W-S-S-C-S-R-S-C-G- (SEQ ID No. 8).

7. Peptide according to one of Claims 1 to 5 of formula:

35 Y-W-S-A₁-C-S-A₂-C-G-Z (SEQ ID No. 9)

in which Y and Z constitute the N- and C-terminal ends of the peptide, or comprise amino acid chains having

less than 6 amino acids, or comprise chains of compounds which are not amino acids.

8. Pharmaceutical composition comprising at least one peptide according to one of Claims 1 to 7 and a pharmaceutically acceptable vehicle.

9. Use of a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

-W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

10 -W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

-W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

15 -W-S-Q-C-S-V-Y-C-G

-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N,

for the manufacture of a medicine intended for the regeneration of the nervous system cells.

20 10. Use of a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

-W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

25 -W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

-W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

-W-S-Q-C-S-V-Y-C-G

30 -T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N,

for the manufacture of a medicine intended for the treatment of neurodegenerative diseases.

35 11. Use of a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

-W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

-W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

5 -W-S-Q-C-S-V-Y-C-G

-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N,

for the manufacture of a medicine intended for the treatment of pathological conditions and traumas

10 requiring regeneration of the nervous system cells and more particularly of their synaptic outgrowths.

12. Use of a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

15 -W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

20 -W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

-W-S-Q-C-S-V-Y-C-G

-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N,

for the manufacture of a medicine intended for the 25 treatment of neuroblastomas.

13. Additive for the cellular cultures of nerve cells, characterized in that it comprises a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

30 -W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)

-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)

-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)

-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R

-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R

35 -W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R

-W-S-Q-C-S-V-Y-C-G

-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N

- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N.

14. Cellular expression vector, characterized in that it comprises a nucleic acid sequence expressing a peptide selected from the peptides according to one of Claims 1 to 7 and the peptides having the sequence:

5 -W-S-P-C-S-V-T-C-G- (SEQ ID No. 2)
-W-S-S-C-S-V-T-C-G- (SEQ ID No. 3)
-W-S-Q-C-S-V-T-C-G- (SEQ ID No. 4)
-W-S-P-W-S-E-W-T-S-C-S-T-S-C-G-N-G-I-Q-Q-R-G-R
-W-S-H-W-S-P-W-S-S-C-S-V-T-C-G-D-G-V-I-T-R-I-R
10 -W-G-P-W-S-P-W-D-I-C-S-V-T-C-G-G-G-V-Q-K-R-S-R
-W-S-Q-C-S-V-Y-C-G
-T-E-W-S-A-C-S-K-S-C-G-M-G-F-S-T-R-V-T-N-R-N
- or T-E-W-S-A-C-S-K-T-C-G-M-G-I-S-T-R-V-T-N-D-N.

15. Cellular expression vector according to Claim 14, characterized in that it comprises a sequence encoding the peptide of sequence SEQ ID No. 8.

DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below next to my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

NOVEL PEPTIDES AND POLYPEPTIDES USEFUL FOR REGENERATING THE NERVOUS SYSTEM

the specification of which is attached hereto unless the following box is checked:

was filed on 16 JULY 1998 as United States Application Number or PCT International Application Number PCT/FR98/01556 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56.

I hereby claim foreign priority benefits under Title 35, United States Code, § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States, listed below and have also identified below any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

NUMBER	COUNTRY	DAY/MONTH/YEAR FILED	PRIORITY CLAIMED
97/09016	FRANCE	16/JULY/1997	YES

I hereby claim the benefit under Title 35, United States Code § 119(e) of any United States provisional application(s) listed below.

APPLICATION NO.	FILING DATE

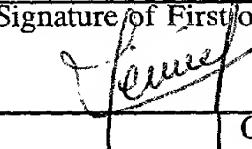
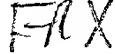
I hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States or PCT International application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I acknowledge the duty to disclose information which is known by me to be material to patentability as defined in Title 37, Code of Federal Regulations § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application:

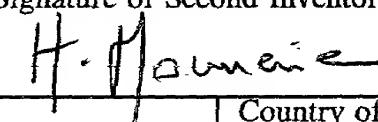
APPLICATION SERIAL NO.	FILING DATE	STATUS: PATENTED, PENDING, ABANDONED

I hereby appoint as my attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Stephen A. Bent, Reg. No. 29,768; David A. Blumenthal, Reg. No. 26,257; William T. Ellis, Reg. No. 26,874; John J. Feldhaus, Reg. No. 28,822; Patricia D. Granados, Reg. No. 33,683; John P. Isaacson, Reg. No. 33,715; Donald D. Jeffery, Reg. No. 19,980; Eugene M. Lee, Reg. No. 32,039; Richard Linn, Reg. No. 25,144; Peter G. Mack, Reg. No. 26,001; Brian J. McNamara, Reg. No. 32,789; Sybil Meloy, Reg. No. 22,749; George E. Quillin, Reg. No. 32,792; Colin G. Sandercock, Reg. No. 31,298; Bernhard D. Saxe, Reg. No. 28,665; Charles F. Schill, Reg. No. 27,590; Richard L. Schwaab, Reg. No. 25,479; Arthur Schwartz, Reg. No. 22,115; Harold C. Wegner, Reg. No. 25,258.

Address all correspondence to FOLEY & LARDNER, Washington Harbour, 3000 K Street, N.W., Suite 500, P.O. Box 25696, Washington, D.C. 20007-8696. Address telephone communications to Patricia D. Granados at (202) 672-5300.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor <u>MEINIEL Annie</u>	Signature of First or Sole Inventor 	Date February 2, 2000
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Residence Address	Country of Citizenship	
Post Office Address		

Full Name of Fifth Inventor	Signature of Fifth Inventor	Date
Residence Address	Country of Citizenship	
Post Office Address		

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: UNIVERSITE D'AUVERGNE
- (B) STREET: 49 BOULEVARD FRANCOIS MITTERAND
- (C) CITY: CLERMONT-FERRAND
- (E) COUNTRY: FRANCE
- (F) POSTAL CODE: 63000

(ii) TITLE OF THE INVENTION: NOVEL PEPTIDES AND
POLYPEPTIDES USEFUL FOR REGENERATING THE
NERVOUS SYSTEM

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER READABLE FORM:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: PatentIn Release #1.0,
Version #1.30 (EPO)

(vi) DATA ON THE PREVIOUS APPLICATION:

- (A) APPLICATION NUMBER: FR 9709016
- (B) FILING DATE: 16-JUL-1997

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 3
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Trp Ser Xaa Cys Ser Xaa Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Trp Ser Pro Cys Ser Val Thr Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Trp Ser Ser Cys Ser Val Thr Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Trp Ser Gln Cys Ser Val Thr Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 3
- (D) OTHER INFORMATION: Xaa means Pro or X₁-W-X₂-X₃ where X₁, X₂ and X₃ are chosen from G, S and C.

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Trp Ser Xaa Cys Ser Xaa Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 3
- (D) OTHER INFORMATION: Xaa means X_1 -W-S- X_3 with X_1 and X_3 chosen from G, S and C

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Trp Ser Xaa Cys Ser Xaa Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 3
- (D) OTHER INFORMATION: Xaa chosen from G,
S and C

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 6
- (D) OTHER INFORMATION: Xaa chosen from G,
S and C

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 9
- (D) OTHER INFORMATION: Xaa means amino
acid sequences comprising from 1 to 5 amino acids.

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Trp Ser Xaa Trp Ser Xaa Cys Ser Xaa Cys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single

REPLACEMENT PAGE (RULE 26)

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Trp Ser Gly Trp Ser Ser Cys Ser Arg Ser Cys Gly
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 10 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 1

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 4
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 7
- (D) OTHER INFORMATION: Xaa means amino acid sequences comprising from 1 to 5 amino acids.

(ix) FEATURE:

- (A) NAME/KEY: specific feature
- (B) LOCATION: 10

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Xaa Trp Ser Xaa Cys Ser Xaa Cys Gly Xaa
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 36 base pairs
- (B) TYPE: nucleotide
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

TGGWSNGGNT GGWSNWSNTG YWSNMGNWSN TGYGGN

36

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 9 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Trp Gly Pro Cys Ser Val Ser Cys Gly
1 5

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 amino acids
- (B) TYPE: amino acid

- (C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Asp Cys Lys Asp Gly Ser Asp Glu
1 5

(2) INFORMATION FOR SEQ ID NO: 13:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 4 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Arg Lys Ala Arg
1

(2) INFORMATION FOR SEQ ID NO: 14:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 12 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear
- (ii) MOLECULE TYPE: peptide

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:
Ser Ser Cys Arg Ser Gly Cys Trp Gly Ser Ser Trp
1 5 10